EPR Studies of Radical Production In Vivo by Lypopolysaccharide. Potential Role of Iron Mobilized from Iron-nitrosyl Complexes

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Although oxidative stress has been implicated in septic shock, there is little evidence for the in vivo formation of radicals other than nitric oxide [1,2]. Here we used low temperature EPR and EPR spin trapping to follow radical formation (2, 6 and 24h) after administration of LPS (0.25 mg; ip) in rats; the animals were further treated with DMSO (1mL/Kg, ip) and POBN (1g/Kg, ip) for 1-3 h before collection and EPR analysis of blood, liver and bile samples. Production of Hb-NO complexes in whole blood followed the previously reported kinetics with maximum levels at 6h and undetectable levels at 24 hs. In parallel with increases in Hb-NO levels, the blood also showed increases in the iron III (g~6) levels, probably as methemoglobin. No clear detection of nitrosyl complexes was possible using low temperature EPR of perfused liver of rats treated with 0.25 mg LPS. In the bile, it was possible to detect POBN radical adducts whose levels were significantly higher than those of the corresponding controls only 24 hs after LPS administration. Administration of DMSO was required to detect significant POBN radical adduct levels in the bile samples. Replacement of DMSO by ^{13}C-DMSO led to the detection of a 12 line EPR spectrum whose computer simulation indicated the presence of two radical adducts, POBN-^{13}C-methyl (a_N= 15.99 G; a_H= 2.88 G and a_{13C}= 4.80 G) and POBN-lipid (a_N= 15.66 G; a_H= 2.55 G) in relative yields of 46 and 54%, respectively. Administration of desferioxamine (0.5 g/Kg, ip) strongly inhibited (> 80%) the signal of the POBN radical adducts detected in the bile 24 hs after LPS administration. Concurrently with this inhibition, it was possible to detect the desferioxamine-iron (III) complex by low temperature EPR of the bile samples. At this point, it is not clear why DMSO administration was required to detect significant levels of POBN-lipid adducts in the bile. The results showed that production of secondary radicals triggered by LPS administration to rats is delayed in regard to maximum NO synthesis and is iron (III)-dependent. Most probably, repair or removal of intracellular iron-nitrosyl complexes led to mobilization of redox active iron to participate in Fenton chemistry. The hydroxyl radicals produced then react with administered DMSO to generate the radical adducts detected in the bile.

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